USDA ARS National Animal Germplasm Program

Ram Semen Collection, Processing and Cryopreservation Protocol for Laparoscopic Insemination

Semen collection and processing:

Collect semen from sexually mature rams via an artificial vagina (preferred) or electroejaculation.

Inspect sample to ensure it is free of urine and other contaminants.

Determine the sperm concentration and motility using spectrophotometry and a Hamilton Thorne motility analyzer (Beverly, MA), respectively (at least 5 fields of analysis and 500 sperm) or microscopy and a hemocytometer.

Dilute the samples using a 1-step dilution, *slowly*, in a 15 or 50 mL tube to 200 x 10^6 sperm/mL with 37 °C Tris-egg yolk Semen Cryopreservation Medium (see recipe below).

Cool samples to 5°C over 2 to 2.5 hours and load into 0.5 mL straws.

Semen cryopreservation and thawing:

Programmable freezer: Freeze samples using a programmable freezer (e.g. the Cryo Bio System Mini Digitcool UJ400, IMV Corporation, Minneapolis, MN) with the following curve: 5 °C to -5 °C at -4 °C per minute, -5 °C to -110 °C at -25 °C per minute, -110 °C to -140 °C at -35 °C per minute.

-OR-

Box freezing: Samples are placed on a rack and frozen in liquid nitrogen vapor (4.5 cm above liquid nitrogen) for 10 min.

Plunge into liquid nitrogen for storage.

Thaw samples for 30 seconds in a 37 °C water bath and analyze for motility as described previously.

Laparoscopic insemination:

Synchronize the estrous cycles of ewes using:

Sponges for 14 days (e.g. Chronogest CR containing 40 mg fluorogestone acetate, Intervet, Milton Keynes, UK) followed by PMSG (400 IU, i.m.; total volume = 4mL from an 18 gauge needle; single injection) administered 48 hours prior to sponge removal;

-OR-

CIDRs (e.g. 0.3 g progesterone in an inert silicone elastomer for 12 days; Pfizer Animal Health, New York, NY) followed by PMSG (400 IU, i.m.; total volume = 4mL from an 18 gauge needle; single injection) administered 24 hours prior to, or at the time of sponge removal;

Restrain ewes in dorsal recumbency and inseminate laparoscopically 56 hours after CIDR removal.

Recipes:

Semen Cryopreservation Medium Recipe from Sanchez-Partida et al., 1998:

300 mM Tris
28 mM glucose
95 mM citric acid
5% glycerol (by volume)
15% egg yolk
1 mg/mL streptomycin sulfate
0.06 mg/mL benzylpenicillin

Reference:

Sanchez-Partida, L.G., Setchell, B.P., and Maxwell, W.M.C. Effect of compatible solutes and diluent composition on the post-thaw motility of ram sperm. Reprod. Fertil. Dev. 1975; 10, 347-357.

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